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PATHOGENESIS OF ACUTE TULAREMIA IN THE RABBIT

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PATHOGENESIS OF ACUTE TULAREMIA IN THE RABBIT

Robert L. Schricker

Medical Bacteriology Division
DIRECTOR OF BIOLOGICAL RESEARCH

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ABSTRACT

Signs, bacterial multiplication and dissemination, and pathologic changes were correlated following intracutaneous inoculation of rabbits with 10^3 cells of Pasteurella tularensis SCHU S4. The infectious process could be divided into four phases: (i) adaptation, (ii) regional infection, (iii) hematogenic dissemination and focal spread, and (iv) septicemia. The organisms became established at the inoculation site during the first 8 hours; they spread to regional lymph nodes and multiplied rapidly during the 8th to 30th hours. Clinical disease coincided with generalization of the process and early pathologic changes. Septicemia was marked by impairment of organ functions, rapid bacterial proliferation and metastasis, progressive anatomic pathologic changes, and terminated in death 103 to 145 hours after inoculation. Bacterial populations per organ in regional lymph nodes, liver, spleen, and lungs were between 10^7 and 10^9 at time of death and did not differ appreciably among animals dying as early as 103 or as late as 145 hours after inoculation. Icterus that developed during the septicemic period resulted primarily from hepatocellular damage.

I. INTRODUCTION

The domestic rabbit* has been used extensively in studies of tularemia,[1-3] but no detailed studies have been reported on the correlation of signs, bacterial multiplication and dissemination, and pathologic changes occurring during the course of the disease in the highly susceptible rabbit.

II. MATERIALS AND METHODS

This study was conducted in three parts:

a) Serial observations - groups of 3 rabbits were inoculated intracutaneously in a hind foot with 10^3 cells of highly virulent *Pasteurella tularensis* SCHU S4, signs were observed, rabbits were necropsied at intervals after inoculation, bacterial populations were assayed, and anatomic pathologic changes were observed,

b) Clinical pathology - groups of 10 rabbits were bled at intervals after inoculation and values for the following were determined; hemoglobin, hematocrit, bilirubin, thymol and zinc sulfate turbidities, serum glutamic oxalacetic transaminase (SGO-T), serum glutamic pyruvic transaminase (SGP-T), total serum protein, urine urobilinogen, icterus index, and erythrocyte sedimentation rate (ESR);

c) Time to death - 10 rabbits were inoculated and bacterial populations in tissues were determined at time of death. A description of histopathologic changes will not be presented in this paper.

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* In conducting the research reported herein the investigators adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

III. RESULTS AND DISCUSSION

The disease could be divided into four phases: (i) adaptation, (ii) regional infection, (iii) hematogenic dissemination and focal spread, and (iv) septicemia.

A. ADAPTATION (PHASE I)

The adaption phase consisted of initial penetration and establishment of the organisms at the inoculation site and lasted for about the first 8 hours after inoculation. Animals were asymptomatic, tissues were grossly normal, clinical chemical values were within normal limits, and the number of recoverable organisms at the inoculation site remained approximately constant.

B. REGIONAL INFECTION (PHASE II)

Bacteria disseminated to the regional lymph nodes and multiplied rapidly during the 8th to 30th hour. Animals were asymptomatic, tissues were grossly normal except for slight congestion at the inoculation site near the end of the phase, and clinical chemical values were normal (Figure 1).

Organisms had disseminated to the regional popliteal nodes in 8 hours and to the internal iliac nodes by the 12th hour. During the regional infection bacterial multiplication at the inoculation site was logarithmic (Table 1).

C. HEMATOGENIC DISSEMINATION AND FOCAL SPREAD (PHASE III)

At about 30 hours the infection became systemic (Figure 2). From the lymphatics the organisms entered the blood stream and were phagocytized by cells of the reticuloendothelial system. Rectal temperatures varied from 104 to 108 F, animals lost about 5% of their body weight, the local inflammatory reaction at the inoculation site that appeared at 30 hours increased, the popliteal lymph nodes were enlarged by 48 hours, and the animals exhibited pain on palpation of the inoculated leg.

Grossly, the inoculation sites were hemorrhagic. The popliteals were about twice normal size, hemorrhagic, and showed miliary necrotic foci. Livers were congested, and spleens were about 1½ times normal size and congested.

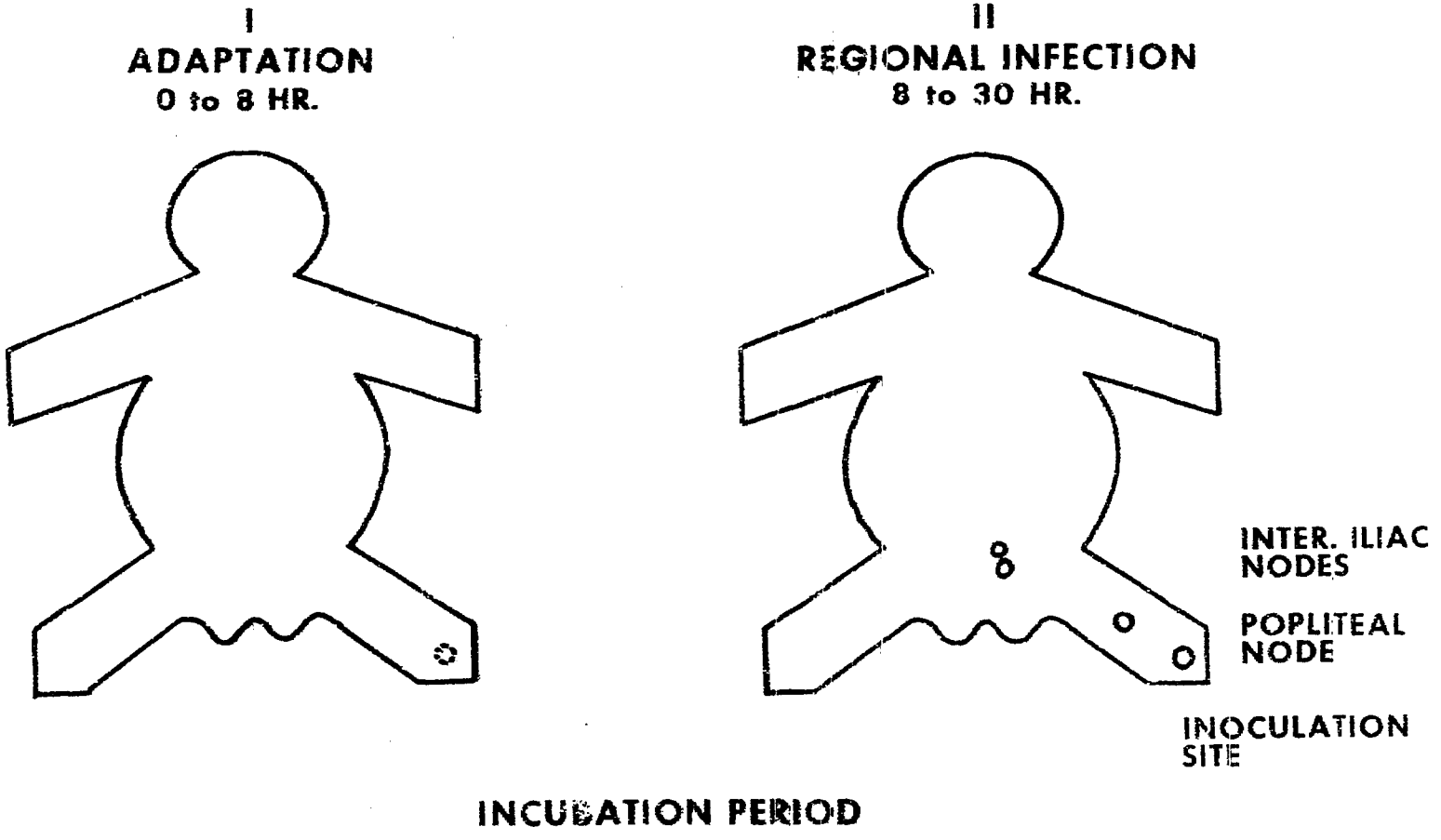

Figure 1. Phases of Tularemia in the Rabbit.

TABLE 1. MEAN POPULATIONS OF PASTEURELLA TULARENSIS IN RABBITS AFTER INTRACUTANEOUS INOCULATION

Phase	Time	Skin Site, per g	Popliteal, per node	Inter. Iliac, per nodes	Liver, per organ	Spleen, per organ	Lung, per organ	Blood, per ml
I	5 min	3×10^2	0	0	0	0	0	0
	3 hr	5×10	0	0	0	0	0	0
	5 hr	2×10^2	0	0	0	0	0	0
II	8 hr	1×10^3	1×10	0	0	0	0	0
	12 hr	3×10^3	2×10	2×10	0	0	0	0
	24 hr	1×10^5	3×10^2	5×10^2	0	0	0	0
	30 hr	1×10^5	3×10^4	9×10^3	5×10^3	3×10^2	0	0
III	36 hr	8×10^6	3×10^5	2×10^4	4×10^3	1×10^3	3×10^4	1×10
	72 hr	1×10^7	7×10^7	1×10^7	1×10^7	3×10^7	3×10^8	6×10
IV	120 hr	1×10^8	8×10^7	2×10^8	2×10^8	6×10^9	4×10^8	3×10^3
	144 hr	3×10^7	2×10^7	5×10^7	2×10^9	5×10^8	2×10^8	4×10^3

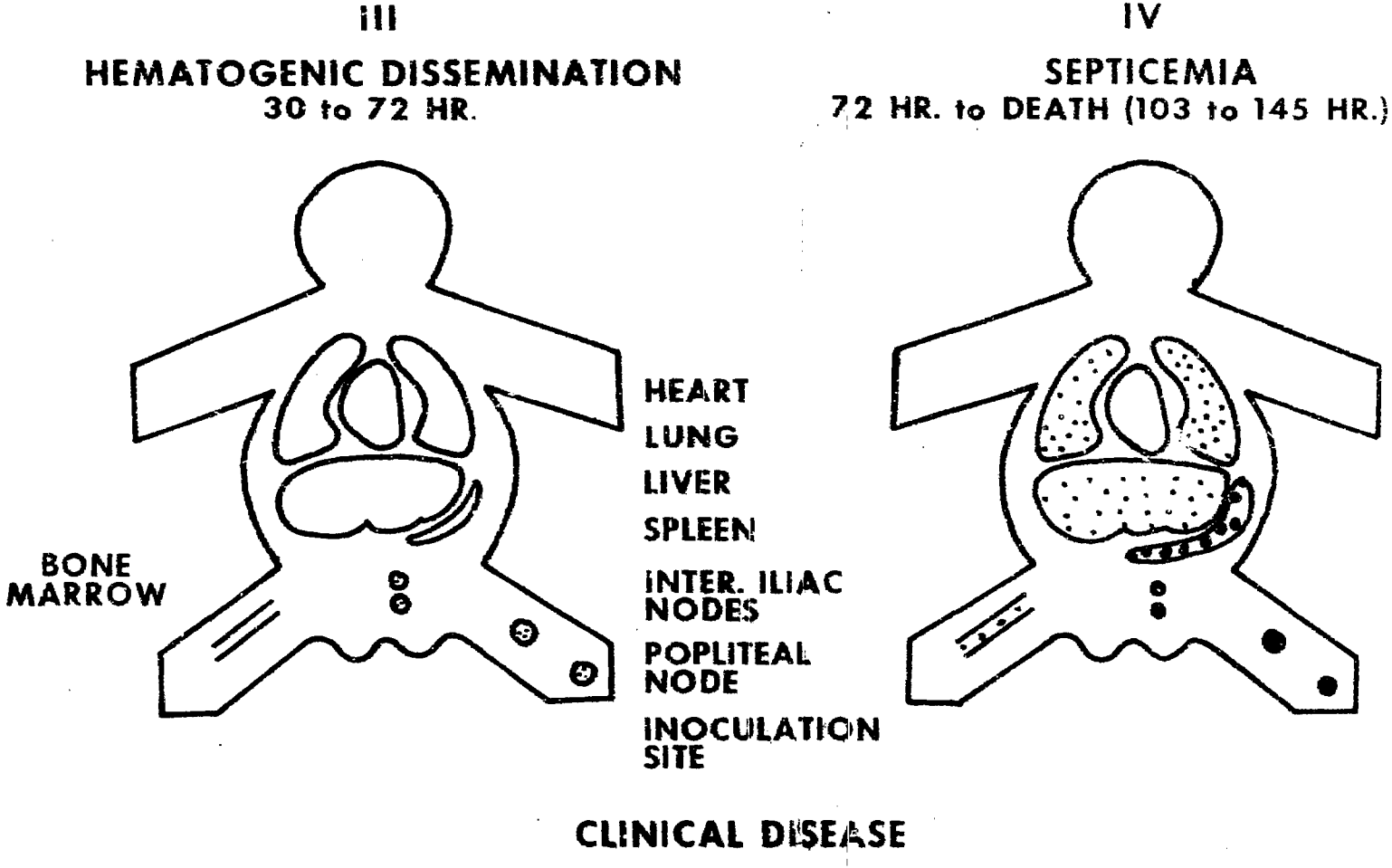

Figure 2. Phases of Tularemia in the Rabbit.

Organisms were first isolated from the liver and spleen at 30 hours. Dissemination to these organs was necessarily through the blood stream, although organisms were not cultured from the heart blood till 6 hours later (36 hours), indicating that the bacteremia was probably transient and consisted of small numbers of cells. Organisms were first isolated from the lungs and femoral bone marrow at 36 hours. Bacterial multiplication at the inoculation site had passed through the log phase by the 36th hour, doubling time was about 2 hours. Bacterial populations in lymph nodes, liver, spleen, and bone marrow multiplied rapidly with doubling times of 2 to 2.8 hours. The blood showed no increase in bacterial concentration during this period.

Clinical chemical values were normal except for an increased ESR that occurred about 12 hours after the infection had become systemic and increased as the disease progressed.

D. SEPTICEMIA (PHASE IV)

Onset of septicemia was marked primarily by abnormal clinical chemical values at 72 hours and terminated in death 103 to 145 hours after inoculation (Table 2). During this period rectal temperatures were 106 to 108 F; weight loss was about 20% at time of death. Animals became progressively more listless, dyspnic, and prostrate near death.

Grossly, the inoculation sites were ulcerated, and caseous areas were observed in the subcutis. The regional lymph nodes showed subtotal caseous necrosis, spleens were about 2½ times normal size with subtotal lesions consisting of coalesced caseous areas. Miliary caseous foci were scattered throughout the liver, lungs, and bone marrow. Icterus was observed in some animals.

Maximum mean bacterial populations were reached in all tissues sampled except blood at about 120 hours and remained constant or decreased at 144 hours (Table 1). This suggested an adverse alteration in the bacterial environment that may have resulted from an increase in toxic metabolic products or an exhaustion of nutrients. P. tularensis produces large quantities of ammonia from amino acids in artificial media and requires strong buffers for cultivation.[4,5] Paper chromatographic studies of serum

TABLE 2. MEAN SERUM VALUES AFTER INTRACUTANEOUS INOCULATION OF RABBITS WITH *PASTEURELLA TULARENSIS*

Phase	Time, hr	Icterus Index	Bilirubin, mg/100 ml		Thymol Turbidity, a/	Transaminase, b/		Erythrocyte Volume, %
			Conjugated	Free		SGO-T	SGP-T	
I, II, III	Preinoc.	5	0	0.6	1	31	36	43
	24	6	0	1.1	1	46	41	41
	48	5	0	0.9	1	26	31	41
	72	9	0.2	0.8	4	105	73	42
IV	144	19	2.6	1.7	7	153	65	35

a. Maclagan units.
b. Sigma - Frankel units.

from infected rats have shown a depletion of amino acids;[6] in vitro infections of rat lymph node slices have shown rapid depletion of cysteine accompanied by decreased tissue respiration.[7] The small numbers of organisms cultured from the blood probably resulted from poor growth in the blood, because P. tularensis is a cytotropic organism and grows best at lower than atmospheric oxygen tension. The time-to-death study showed that death occurred when bacterial populations reached 10^7 to 10^9 in most organs; animals died from 103 to 145 hours after inoculation. This similarity of P. tularensis populations in tissues assayed at death suggests a relationship between the number of organisms in the tissues and time to death.

Icterus index, serum bilirubin, urine urobilinogen, thymol and zinc sulfate turbidities, SGO-T, and SGP-T determinations showed elevated values beginning about 72 hours after inoculation; packed erythrocyte volumes were moderately below normal about 48 hours later (120 hours) (Table 2). Hemoglobin and total serum protein levels remained within preinfection levels throughout the course of the disease. The increase in conjugated bilirubin, SGP-T, and urine urobilinogen, and to some extent the SGO-T and zinc sulfate and thymol turbidities indicated that the icterus was due to hepatocellular damage rather than to hemolysis or extrahepatic obstruction of the bile ducts.

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